Supercritical carbon dioxide extraction ($SCCO_2$) of Chiba seed (Psoralea corylifolia) and LC-MS characterization of the of the extract

Manohar Balaraman, Usha Rani Ganni , and Udaya Sankar Kadimi*

(Food Engineering, Central Food Technological Research Institute, Mysore 570020, India)
E-Mail: udaya@cftri.res.in and Fax:+91-821-2517233

Abstract:

The SC CO_2 extracts of Chiba Seeds (*Psoralea corylifolia*) are obtained at pressures (18, 28 and 38 MPa) and at three temperature (40^o,50^o and 60^oC) to obtain extracts rich in bakuchiol to optimize the conditions of extraction. The extracts are analyzed for bioactive components bakuchiol, psorlene isopsoralene. Bakuchiol is the main component of interest is obtained by SC CO_2 extraction in substantial quantities (>50%) and the extraction by traditional solvent extraction results in poor recovery as it was found to be thermally labile. Supercritical fluid carbon dioxide extraction (SCFE CO_2), a novel method for extraction, more relevant for obtaining thermally labile natural products, is cost effective and eliminates toxic organic waste. The SC-CO_2 extracts were investigated for the occurrence of different components bakuchiol, psoralen, isopsoralen, corylin, psoralidin, bavachromene, isobavachalcone, corylifol A, bavachlcone, Brosimachutin G, bakuchiol and was confirmed by high-performance liquid chromatography (HPLC) and by Liquid chromatography – Mass Spectrometry (LC-MS) in electron spray ionization (ESI) negative mode. The structures of bakuchiol, sporalen and isosporalen are confirmed by 500 MHz NMR studies. Most of the compounds has reported to have high degree of antibacterial activities and anticancer properties.
Key words: psoralea corylifolia, supercritical carbon dioxide, bakuchiol, psoralen, extraction

INTRODUCTION:
Psoralea corylifolia L., (Chiba seed) is an ancient medicinal plant of India and China and widely used in Traditional Chinese Medicine (TCM) for the treatment of various kinds of disorders such as asthma, cough, nephritis, vitiligo and also some skin diseases, e.g., leucoderma, psoriasis and leprosy [1,2].The seeds are refrigerant, alternative, laxative, antipyretic, anthelmintic [3], alexiteric and good for heart troubles. Chemical constituents such as coumarins, flavonoids and meroterpene phenols were isolated from this plant and some of which exhibited antitumour [4], antibacterial and antivirus activities and can affect metabolism of some remedy [5], antifungal activity [6,7], broadening coronary artery and estrogen-like activities [8]. Meroterpene phenol known as bakuchiol is responsible for antioxidant [9], antiplatelet [10, DNA polymerase and topoisomerase II inhibition [11] and osteoblastic proliferation stimulating [12] activities. The seed extract of *P. corylifolia* showed inhibition of antigen-induced degranuatlion in RBL-2H3 cells [13].
Conventionally, isolation of plant-derived compounds are carried out with organic solvent and Supercritical carbon dioxide (SC-CO_2) extraction offers a promising separation technology for

thermally labile natural products and recently investigated for extraction of several active compounds from natural materials. The most important advantage of utilizing SC-CO_2 is the easy separation of the solvent from the extracted material, nontoxic and it operates at ambient temperature that does not affect the heat sensitive compounds. Further, SC-CO_2 provides lower mass transfer resistance than those in conventional separation processes [14,15].
The purpose of this study was to investigate SC-CO_2 extraction from the seeds of *Psoralea corylifolia* as an alternative for conventional solvent extraction processes and identification of the compounds in the SC-CO_2 extract.

MATERIALS AND METHODS

Materials: Seeds of *Posralea corylifolia* (Leguminosae) were supplied by M/s Kumar Organics Private Limited, Bangalore. Carbon dioxide gas was supplied by Kiran Corporation, Mysore. Acetonitirle was HPLC grade and Water was purified by a Milli-Q system. Hexane was analytical grade.
SC-CO_2 EXTRACTION: The NOVA SWISS Ex 4.0 high pressure extraction unit was used for extraction of *P.corylifolia*. The unit equipped with a compressor, high-pressure extractor, separator and heating arrangements for the extractor and separator and the carbon dioxide can be receompressed back and re-circulated. Thermocouple-based digital indicating system and pressure transducers to measure temperature and pressure and endowed with flow measurement system [14]. Experiments were conducted at pressures (18, 28 and 38 MPa) and at three temperature (40^o,50^o and 60^oC) to obtain extracts and monitored for rate of extraction
HEXANE EXTRACTION:The seed powder was extracted in hexane by using Soxtec Extraction system (Tecator, Switzerland). About 5gms of seed powder was accurately weighed and initially boiled in hexane for a period of 30min. and later rinsed for two hours. The extract is made into 50 ml with hexane and the percentage extract was obtained by evaporating to dryness 5 ml portions in triplicate and the weight obtained was expressed percentage extract on dry weight basis.
HPLC ANALYSIS: The extracts obtained by Hexane extraction (at ambient and subdued light) and carbon dioxide extractions were analyzed by HPLC. The HPLC system consisted of delivery pumps (Shimadzu LC-10A, Japan), a UV detector and a manual injector valve with a 0.02ml sample loop and analysis conducted with a modified method [16].
Chromatographic separation was carried out at room temperature using a Phenomenex C18 analytical column (250mm x 4.60mm, 5µm). The mobile phase used for the analysis of Chiba seed oil was an isocratic elution with Acetonitrile-water (70:30) at a flow rate of 1.0ml/min. The concentration of the sample was 1mg/ml and injection volume was 20µl. The effluent was detected at 261nm.The results were obtained within 30minutes.
LC-MS ANALYSIS:The sample solutions were analyzed by Liquid Chromatography - Mass Spectrometry (Waters 2996, photodiode array detector, USA) technique. The mass spectrometry detector was equipped with an ESI (electrospary ionization) source. The ionization mode was negative. Liquid chromatography conditions were same as indicated above. The mass spectrometry conditions were as follows: Capillary, -3.00KV; Cone, 100; Source temperature, 120°C; Dissolvation temperature, 300°C; Cone gas, 50L/hr; Dissolvation, 500L/hr; scan range, m/z 100-800 with a scan speed of 1000amu/sec. A peak thresh hold of 1% identity was applied to the mass spectra.

Nuclear Magnetic Resonance (NMR) Spectrum: The purified samples were analyzed by ^{1}H and ^{13}C NMR Spectra of Bruker-500MHz spectrometer. The NMR spectral scans are carried out in duteriumchloride ($CDCl_3$) solution.

Antioxidant activity measurement: Antioxidant activity of bakuchiol and bakuchiol glycosides were determined by DPPH (2,2 diphenyl-1-picryl hydrazyl) radical scavenging method [17]. The reaction mixture contained 0.1 mL of test sample (5-10mM) and 1.0 mL of DPPH (0.36 mM) with the final volume adjusted to 2.0 mL of 0.1M Tris HCl buffer (pH 7.4). The reaction mixture was incubated at room temperature for 20 minutes in the dark and the antioxidant activity was determined by monitoring the decrease in absorbance at 517 nm on an UV-Visible spectrophotometer (Shimadzu, UV 1601). Butylated hydroxy anisole (BHA- 5.6mM) was used as the positive control. IC_{50} value for the antioxidant activity was expressed as the concentration of the glycoside corresponding to 50% decrease in absorbance value of DPPH from a plot of decrease in absorbance versus concentration of the glycoside. Error in activity measurements is ± 5%.

S.No.	Pressure M Pa	Temp, ^{o}C	Yield of extract (%) [a]	Bakuchiol (%)
1	18	40	22.2	48.1
2		50	33.1	52.3
3		60	36.8	56.1
4	28	40	59.0	60.4
5		50	45.2	52.3
6		60	38.2	54.2
7	38	40	54.2	0.61
8		50	50.2	0.40
9		60	56.2	0.60

Table 1. SC CO2 extraction of chiba seed powder

RESUTLS AND DISCUSSION:

Table 1 provide the yield of extract on extraction of chiba seed for different pressures and temperature for same amount of solvent to material ratio and also the content of bakuchiol of the extract.The yield of the extract is proportional to the density of carbon dioxide used, while the bakuchiol content for the extracts obtained at $60^{o}C$ is low. The bakuchiol tend to cyclize in presence of p-toluene sulfonic acid [18], the HPLC and mass studies indicate the cyclization bakuchiol as per scheme indicated below (Figure 1).

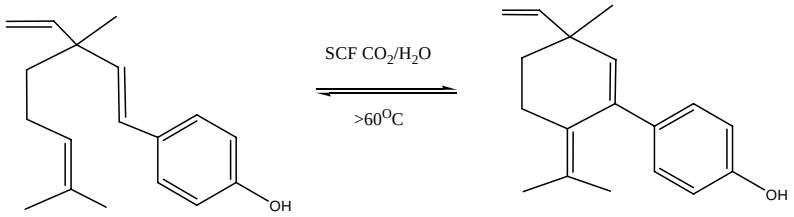


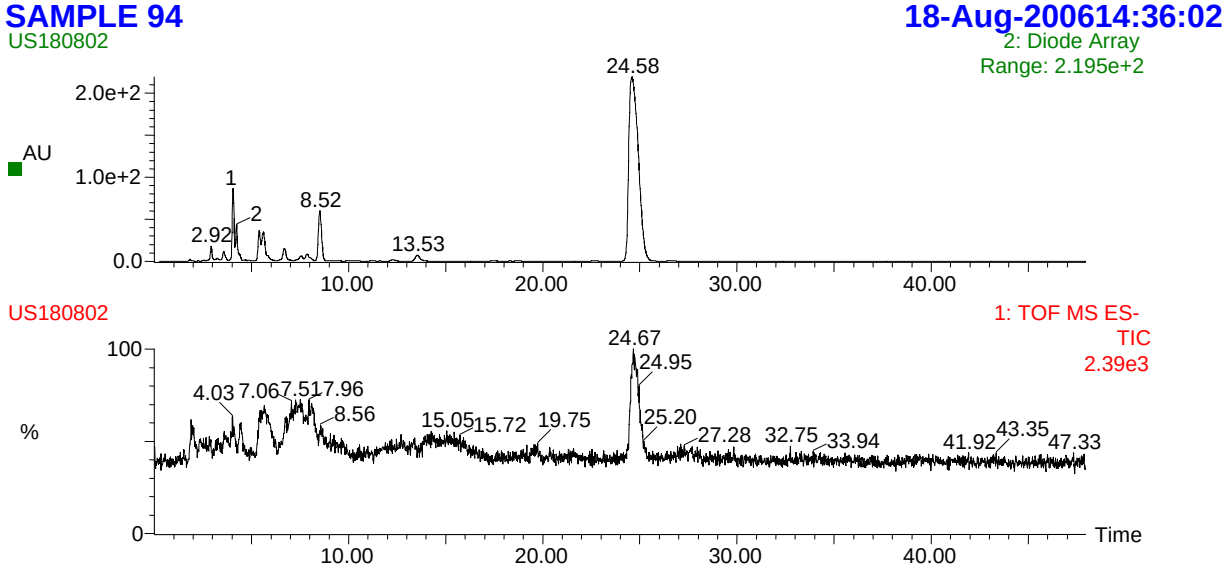
Figure 1. Cycliation of bakuchiol

The Chiba extract produced through the SC-CO_2 process was analyzed by HPLC and LC-MS.

Fig 2 shows the chromatograph of SC-CO_2 sample along with mass spectra. The same sample was analyzed by LC-MS. In an ESI-MS experiment, we obtained molecular weight of each peak. Comparing with the mass spectral data reported in literatures for the components in *P.corylifolia*, 10 major components were identified. Table 2 shows the components identified by LC-MS analysis along with their mass spectral data. Because the ionization mode was negative, most of the m/z data are [M- H]. Table 2 shows the mass fragmentation o of the individual components. Peak 1 and 2 in ESI (-) - MS data, [M-H] is 185, so the molecular weight of this component is 186. This substance was deduced as psoralen or isopsoralen. Comparing with the spectra of the standard compounds, we confirm peak 1 as

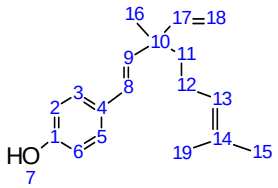
psoralen and peak 2 as isopsoralen. The mass spectrum of peak 3 exhibits molecular ion peak at [M-H] is 319, according to literature data [Guo et al] this peak was identified as corylin. Guo et al [8] separated and identified psoralidin from *P.corylifolia*, as molecular weight was calculated as 336.10, this is quite similar with peak 4 and MS data, and [M-H] is 335, so peak 4 could be identified as psoralidin. By comparing the mass spectrum with the literature data [8] peak 5 was identified as bavachromene. Peak 6 shows molecular ion peak at [M-H] is 323, this is identified as isobavachalcone. Yin et al. [9] separated and identified corylifol A from *P.corylifolia*, and the molecular weight was calculated as 390, which was very similar to ion peak on ESI (-)-MS data,[M-H] is 389 for peak 7, so this peak was preliminary identified as corylifol A. Peak 8 shows mass ion peak at [M-H] is 337, it was identified as bavachalcone. The mass spectrum of peak 9 on ESI (-), [M-H] is 355, which was quite similar with the literature data, so the compound identified as Brosimachutin G. By comparing the mass spectrum of peak 10 with that of bakuchiol, peak 10 was identified as bakuchiol, with its molecular ion peak at [M-H] is 255.

Figure 3: The LC-MS chromatogram of the *P.corylifolia* seed oil.

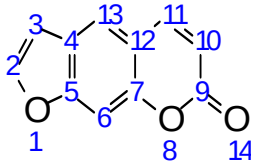


Pure bakuchiol showed an antioxidant activity of 1.24 mM (IC_{50} value) as against 0.029 mM for synthetic antioxidant BHA. Various glycosides of bakuchiol showed antioxidant activities ranging from 1.02 to 2.28 mM.

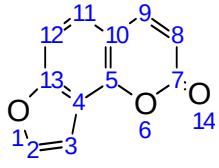
NMR Spectra:



Bakuchiol



Psoralen



Isopsoralen

Further the structure of bakuchiol, psoralen and isopsoralen is further conformed by the NMR spectra. The compounds were conformed by comparing with the literature data as well as predicted data of ^{1}H and ^{13}C NMR data by Cambridge software (http://www.cambridgesoft.com). The integrated data are provided in Table 3 with structures and the coupling constants are expressed in Hz.

Table 2 Mass spectral data of identified compounds

Peak No.	Compound	Molecular formula and Mass	Mass spectral data
1	Psoralen	$C_{11}H_6O_3$, 186	183(100), 184.17(45), 185.19(25), 187.28(26) 189.20(26)
2	Isopsoralen	$C_{11}H_6O_3$, 186	183(100), 184.17(45), 185.19(25), 187.28(26) 189.20(26)
3	Corylin	$C_{20}H_{16}O_4$, 320	317.40(15), 318.36(7), 319.38(100), 319.41(50), 320.39(24), 321.36(12)
4	Psoralidin	$C_{20}H_{16}O_5$, 336	325.46(100), 335.42(45), 335.48(85), 335.61(54), 339.47(80), 340.53(28)
5	Bavachromene	$C_{20}H_{18}O_4$, 322	320.75(18), 321.34(30), 321.36(50), 321.38(100), 321.43(50), 321.49(30), 322.23(18), 322.42(32)
6	Isobavachalcone	$C_{20}H_{20}O_4$, 324	321.38(18), 323.43(40), 324.41(10), 325.46(100), 326.45(25)
7	Corylifol A	$C_{25}H_{26}O_4$, 390	375.51(10), 389.49(80), 389.52(100), 390.50(30), 391.49(10), 401.25(10)
8	Bavachalcone	$C_{21}H_{22}O_4$, 338	325.45(35), 326.45(12), 333.38(20), 335.41(12), 337.46(100), 339.48(50), 339.52(40), 340.53(10)
9	Brosimachutin G	$C_{20}H_{20}O_6$, 356	353.15(100), 353.17(80), 353.20(60), 354.51(30), 355.41(57), 355.51(63), 355.54(47), 355.56(37), 356.46(25), 357.18(32), 357.43(50)
10	Bakuchiol	$C_{18}H_{24}O$, 256	248.29(20), 248.31(30), 253.26(20), 253.30(30), 255.38(75), 255.42(100), 255.43(65), 255.47(40), 261.38(30)

Table 3 ^{1}H and ^{13}C NMR spectra data of purified compounds.

Name of compounds	^{13}C NMR (500Hz)	^{1}H NMR (500Hz)
Bakuchiol	18.1 (C_{19}), 22.7 (C_{12}), 26.4 (C_{15}), 29.2 (C_{16}), 41.0 (C_{10}), 42.2 (C_{11}), 111.5 (C_{18}), 114.7 (C_6), 115.1 (C_2), 124.5 (C_8), 126.2 (C_{13}), 127.1 (C_4), 127.6 (C_5), 128.2 (C_9), 129.8 (C_{14}), 130.7 (C_3), 145.6 (C_{17}), 154.5 (C_1).	1.22 (s, 2H, H-10), 1.50 (d, 1H, J=6, H-16), 1.6 (s, 3H,H-15, H-19), 1.97 (d, 2H, J-9.0, H-12), 5.05 (d, 2H, J=1.0, H-18), 5.07 (OH, J=1.5, H-7), 5.13 (d, 1H, J=1.5, H-13), 5.876 (d, 1H, J=11, H-17), 6.05 (d, 1H, J=16, H-9), 6.25 (d, 1H, J=16, H-8), 6.779 (d, 2H, J=9.0, H-6, H-2), 7.247 (d, 2H, J=8.5, H-3, H-5)
Psoralen	99.556 (C_6), 106.059 (C_3), 114.362 (C_{10}), 115.118 (C_{12}), 119.512 (C_{13}), 124.563 (C_4), 143.708 (C_{11}), 146.585 (C_2), 151.753 (C_7), 156.119 (C_5), 160.650 (C_9)	6.38 (d, 1H, J=10.0,H-10), 6.85 (d, 1H, J=1.0, H-3), 7.487 (s, 1H, H-6), 7.702 (s, 1H, H-11), 7.71 (d, 1H, J=2.0, H-13), 7.809 (d, 1H, J=10.0, H-2)
Isopsoralen	160.655 (C_7), 156.119 (C_{13}), 148.201 (C_5), 146.583 (C_2), 143.707 (C_9), 124.562 (C_4), 123.497 (C_{11}), 115.1 (C_{10}), 114.362 (C_8), 108.471 (C_{12}), 106.056 (C_3)	6.406 (d, 1H, J=7, H-8), 6.847(s, 1H, H-3), 7.14 (d, 1H, J=0.5, H-12), 7.39 (d, 1H, J=8.5, H-11), 7.44 (d, 1H, J=8.5, H-9), 7.82 (d, 1H, J=9.5, H-2)

CONCLUSTIONS: The extraction of chiba seed (*Psoralea Corylifolia L.,)* for obtaining bakuchiol using SCF CO_2 is only possible at temperatures below 60^oC without cyclisation and the components are obtained in better recovery when compared to conventional extraction with structural integrity. The bakuchiol, psoralen, iso-psoralen, corylin, psoralidin, bavachromene, isobavachlcone, corylifol A, bavachlacone, brosimachutin G were identified LC-MS studies and confirmed by their ^1H and ^{13}C NMR spectra. The antioxidant activity of bakuchiol is higher than BHT and most of the compounds identified in the extract showed anti-oxidant activity compared to synthetic BHT.

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